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Focus
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Bacterial diarrhoea diagnosis: faecal culture

General

The definition of acute diarrhoea (Remic 2007) is the excretion of at least three loose or soft stools per day for less than 14 days. In cases of chronic diarrhoea, it is unnecessary to prescribe a faecal culture other than for immunocompromised subjects.

Not all cases of diarrhoea are caused by infection; viruses, yeasts and parasites play a major role in infectious diarrhoea. In effect, the frequency of positive faecal culture results is low, from 0.5 to 14% on pathological stools.

Faecal culture: indications

- **Soft** (taking the shape of the container), loose or bloody **stools.**
- **Hard stools:** only in an epidemiological context (BSLB monitoring, *C. difficile* toxin excretion, etc.). A minimum number of clinical data items are required: adult or child, clinical signs (fever, vomiting), context (possibility of travel, food poisoning, diarrhoea in close family and friends, antibiotic treatment, screening: MRB, toxins, etc.).

Conditions

The sample (quantity: 10-20 g) is collected in a sterile container, with a screw-on lid, or on a swab for infants. The transport should be ‹ 2 hrs; the container may be stored at + 4°C overnight.

- **Direct examination:** Gram staining is not useful. Fresh examination is preferable, for observing leukocytes, red blood cells and bacterial polymorphism (flora equilibrium).
- **Culture:** the inoculation of specific media is essential.

Physiopathology

The source of contamination is usually found in water or food contaminated by an infected subject or a healthy carrier.

Schematically, two mechanisms take place:

- **Toxinogenic action:** abundant watery diarrhoea, choleriform syndrome (case of *V. cholerae, Escherichia coli* in developing countries, *Aeromonas sp., Plesiomonas sp., C. difficile, S. aureus, Bacillus cereux*).
- **Invasive action:** dysenteric syndrome caused by *Salmonella sp., Shigella sp., Campylobacter, Yersinia*.

Clinical-epidemiological contexts

Toxinogenic diarrhoea

- **Entero-toxinogenic *E. coli* (ETEC)**
  The agent involved in holiday diarrhoea, it is particularly prevalent in developing countries and causes choleriform diarrhoea. Testing is performed on explicit request, on return from travel.
- **Entero-pathogenic *E. coli* (EPEC)**
  Responsible for acute or chronic, in some cases bloody and/or febrile, diarrhoea, essentially in infants (neonatology units, childcare centres), but has practically disappeared from industrial countries.
  Testing is performed on explicit request, for children under 3 years of age.
- **Entero-haemorrhagic *E. coli* (EHEC),** recently named Shiga toxin or verotoxin-producing E. coli (STEC)
  Serotype O157:H7 is the most frequent STEC (followed by O26, O55, O91). The main sources of contamination are beef, raw milk and animal products. It is responsible for severe haemorrhagic colitis, haemolytic-ureamic syndrome (HUS), thrombotic thrombocytopenic purpura. It should be tested on bloody stools in children.
- **Cholera**
  Caused by *Vibrio cholerae* (frequent serotypes O1 and O139), it is currently particularly prevalent in Africa and in developing countries. Contamination takes place via water and contaminated food; the cholera toxin excreted in the intestine causes severe diarrhoea and vomiting (loss of water and electrolytes of up to 15 l/day) without fever, after an incubation period ranging from a few hours to a few days.

  A rapid diagnostic test strip has been available since 2006.

- ***Aeromonas sp.***
  Prevalent worldwide. Contamination occurs through drinking water (hot season) and seafood. It causes febrile watery acute diarrhoea and vomiting. In biological terms, it is isolated on blood agar with ampicillin (it is resistant to this antibiotic) or Chromagar®, and is oxidase +.

- ***Plesiomonas sp.***
  Found in water with low salt levels not subject to cold temperatures, it causes choleriform, in some cases serous-bloody, diarrhoea lasting from a few days to 6 months and pseudo-membranous colitis. Contamination occurs by consuming water, raw shellfish or raw fish, swimming in contaminated water, or handling amphibians or reptiles. The media used for its isolation are MacConkey and SS media.

Invasive diarrhoea

- ***Salmonella sp.***
  This is the most frequent cause of acute alimentary bacterial diarrhoea. The incubation period is 12 to 36 hours. The bacteria are transmitted by food (eggs, poultry, minced meat, dairy products, cheese) or by healthy carriers working in the catering industry.

  Salmonella sp gives rise to gastroenteritis with diarrhoea, vomiting, abdominal pains, with spontaneous recovery in 2 to 3 days, but requiring hospitalisation in 25% of cases. The nomenclature was recently amended, designating a pathogenic species in humans, *Salmonella enterica*. The serotype is determined by means of slide agglutination with various antisera of the antigen formula designating serovars from the parietal O, flagella H and capsular Vi antigens (*Typhi, Typhimurium, Enteritidis serovars*, etc.).

- ***Shigella sp.***
  Responsible for bacillary dysentery, endemic in tropical regions. The primary cause of this disease is a lack of hygiene. It is transmitted directly from the infected subject to those in close contact with him/her or indirectly by water and food contaminated by fly dejecta. The four main serological groups (O Ag) are:

  - ***Shigella dysenteriae* group**
    (Latin America, Africa, Asia): 10 serotypes (epidemics).

  - ***Shigella flexneri* group**
    (USA, France, but also endemic): 8 serotypes.

  - ***Shigella boydi* group**
    (rare in USA and France): 15 serotypes.

  - ***Shigella sonnei* group:**
    main cause in industrial countries (1 serotype).

  Infection particularly affects children under 5 years of age. After a brief incubation period, abdominal pains, colic, tenesmus, vomiting and mucous-bloody, purulent, haemorrhagic diarrhoea (up to 100 stools/day) occur. A high fever occurs and the general condition is impaired, with a high risk of dehydration. Antibiotic therapy is compulsory (beware of multi-resistant *S. flexneri* and *S. dysenteriae* serotype 1 strains). Recovery is obtained in a few days but patients may be subject to relapses. The existence of healthy carriers should be noted.

- ***Campylobacter sp.***
  The primary cause of bacterial gastroenteritis, *Campylobacter* infection is cosmopolitan. It is transmitted essentially by food (cross-contamination with raw poultry, but also raw milk or non- or incorrectly chlorinated water).

  The isolation of *Campylobacter* in the laboratory requires the inoculation of a specific agar (Campylosel®) under micro-aerophilic conditions. The test is performed on request, or systematically in the case of loose stools.

  After an incubation period of 2 to 3 days, infection gives rise to loose +/- bloody diarrhoea and abdominal pains, accompanied by systemic signs (fever, tiredness, loss of appetite). The progression is spontaneously favourable. Complications such as bacteraemia are rare (< 1%), and slightly less rare in the case of *C. fetus*.

- ***Yersinia enterocolitica***
  Y. Enterocolitica propagates at low temperatures, in food stored at + 4°C. Transmission occurs from consuming pork, milk, raw vegetables and human-to-human transmission occurs by the faecal-oral route. The bacterium is excreted over a prolonged period in stools after recovery.

  Infection gives rise to febrile gastroenteritis (moderate temperature), watery and sometimes bloody diarrhoea, abdominal pains, vomiting, or mesenteric adenitis.

  The bacterium is to be tested on CIN specific medium incubated at 30°C, using biochemical tests. Cephalothin, ampicillin, ticarcillin and piperacillin resistance is practically systematic.

Faecal culture: interpretation (Rémic 2007)

- The presence of Salmonella spp., Shigella spp., Campylobacter spp. and Yersinia enterocolitica in a standard faecal culture is always pathological.
- The presence of Escherichia coli, even in large quantities, should not be considered to be pathological. Only shiga toxin-excreting enterohaemorrhagic E. coli strains should be considered to be pathogenic. Enteropathogenic E. coli (EPEC) causes acute diarrhoea in children only.
- S. aureus cannot be implicated in acute diarrhoea lasting for more than 24 hours.
- Any bacterium considered to be pathogenic isolated from a faecal culture should be considered to be the cause of the symptoms.
- In France, any collective food poisoning involving more than 2 cases must be reported to the health authorities.

Conclusion

Bacteriological analysis must always include an interpretation, in the form of a report. Failing sufficient clinical data, it may be substituted by a comment summarising the main pathogens, according to the clinical context.

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